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SPONSOR REPORT

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Trace determination of Octyl&Nonyl-phenols, related Ethoxylates and Bisphenol A using on-line SPE and Orbitrap LC-MS/MS

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Purpose: To demonstrate the feasibility of using a Thermo Scientific Focus Hybrid Quadrupole-Orbitrap MS with a Thermo Scientific™ EQuan MAX Plus™ system to meet method detection limits for compliance with EU Water Frame Framework Environmental Quality Standards (EQS) for bisphenol A and key Alkylphenol and related Ethoxylates.

Methods: EQuan on-line SPE High Resolution Accurate Mass using Selected Ion Monitoring (SIM) and Parallel Reaction Monitoring (PRM).

Results:

Component	EQS Surface/ Marine Waters (ng/L)	Required LoD(ng/L)	Achieved LoD* Surface/ Marine/ Ground waters	Achieved LoD* WWTW effluent
NP-1-EO	4800 (1)	480	50	50
NP-2-EO	4800 (1)	480	7	7
NP-3-EO	4800 (1)	480	7	7
OP-1-EO	N/A	N/A	90	140
OP-2-EO	N/A	N/A	4	15
OP-3-EO	N/A	N/A	5	5
4-Nonylphenol (branched)	300 (total NP)	30	15	25
4-n-Nonylphenol			10	20
Bisphenol-A	1400 (2)	140	40	60
4-t-Octylphenol	100/ 10	10/ 1	4	4

(1) Predicted No Effect Concentration
(2) Proposed value

INTRODUCTION

The occurrence and effects of endocrine disrupting compounds (EDCs), particularly mixtures, in aquatic environments is a significant concern[1]. Of the many EDCs, bisphenol A, Alkylphenols and related Ethoxylates are recognised as possessing significant estrogenic potency and risk to freshwater ecosystems and drinking water resources[2]. Due to their environmental significance, the compounds were incorporated into the EU Water Framework Directive[3], with low EQS limits of detection, which present a significant analytical challenge, particularly for routine monitoring methods requiring high throughput.

Current methods generally involve large-volume SPE; normal phase SPE clean up and size exclusion fractionation, derivatisation and GCMS or LCMS determination, which take considerable time, expense, and sampling logistics (Figure 1).

The aim of this work is to assess the feasibility and performance of using a 2.5 mL sample on-line solid phase extraction and a Q Exactive Focus Orbitrap MS, for the determination of the targets at the required EQS MDLs.

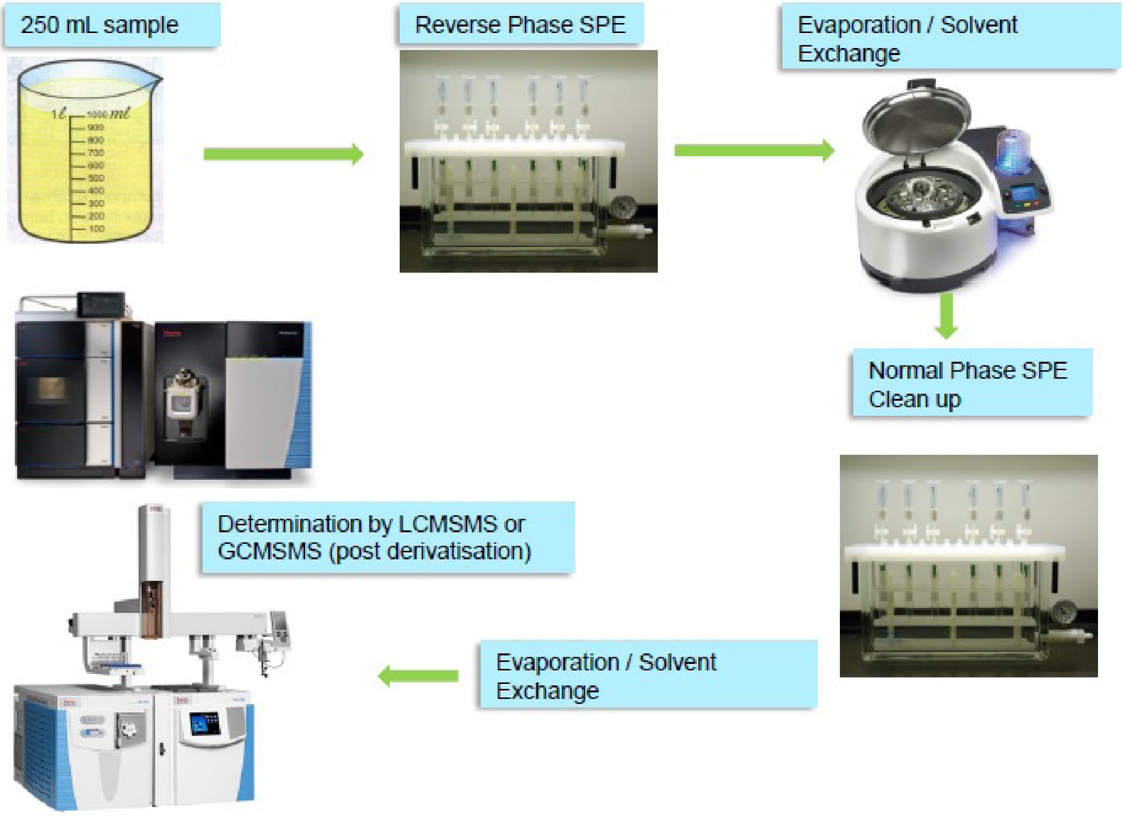


Figure 1. Typical workflow for Bisphenol A, Alkylphenols and related Ethoxylates analysis.

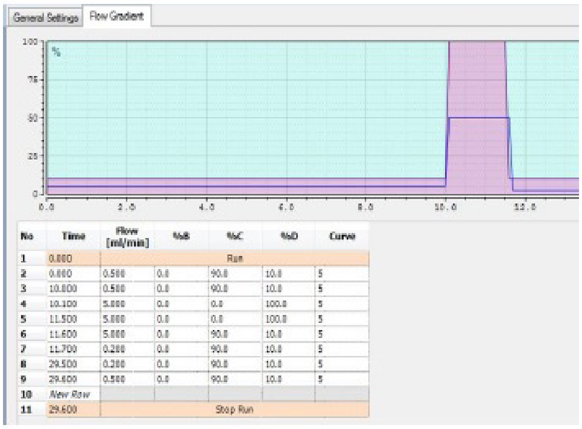
MATERIALS AND METHODS

Liquid Chromatography

Liquid Chromatography separations were carried out on EQuan MAX Plus™ system including binary analytical pump, CTC autosampler, quaternary loading pump and column compartment; see Figure 2. Alkylphenols and Bisphenol A were determined with negative ionisation using PRM; and the ethoxylates determined with positive ionisation using SIM. Two separate runs were carried out.

Loading Conditions:
Column: Thermo Scientific™ Hypersil GOLD™ aQ 12 µm 20 x 2.1
Injection volume: 2.5 mL
Loading rate: 0.5 mL/min
Loading solvent:
C - H_2O (0.1% Formic acid).
D- Methanol (0.1% Formic acid)

Syringe and valve Cleaning Solvents:
1 – Acetonitrile: Acetone: Propan-2-ol:- 70:20:10
2 – Acetonitrile.

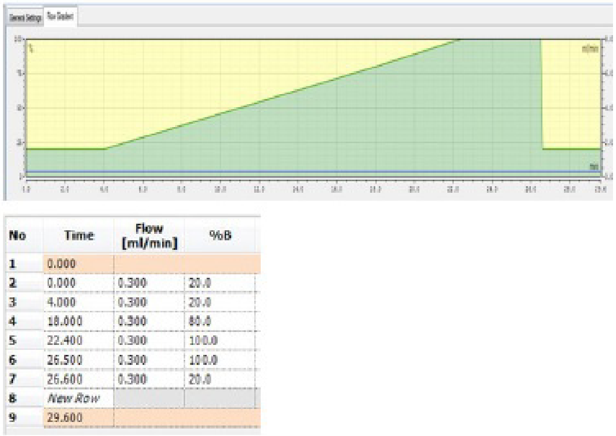


No	Time	Flow [ml/min]	%B	%C	%D	Curve
1	0.000	Run				
2	0.000	0.500	0.0	90.0	10.0	5
3	10.000	0.500	0.0	90.0	10.0	5
4	10.100	5.000	0.0	0.0	100.0	5
5	11.500	5.000	0.0	0.0	100.0	5
6	11.600	5.000	0.0	90.0	10.0	5
7	11.700	0.200	0.0	90.0	10.0	5
8	29.500	0.200	0.0	90.0	10.0	5
9	29.600	0.500	0.0	90.0	10.0	5
10	New Row					
11	29.600	Stop Run				

Analytical Conditions:
Column: Thermo Scientific™ Accucore™ aQ 100 x 2.1, 2.6 µm
Injection volume: 2.5 mL
Flow rate: 0.3 mL/min
Needle Wash Solvent: 50%MeOH & 50% ACN

Mobile phase:
A - H_2O (no buffer)
B – MeOH (no buffer)

Post column infusion: Ammonia solution (1.5% w/v in methanol) 11-21 mins, 20µL/min.



No	Time	Flow [ml/min]	%B
1	0.000		
2	0.000	0.300	20.0
3	4.000	0.300	20.0
4	18.000	0.300	80.0
5	22.400	0.300	100.0
6	26.500	0.300	100.0
7	26.600	0.300	20.0
8	New Row		
9	29.600		

Mass Spectrometry

The MS analysis was performed on a Q Exactive Focus bench-top high resolution mass spectrometer using heated electrospray ionization (HESI). The Alkylphenols and Bisphenol A were determined using Parallel Reaction Monitoring (PRM) where MS/MS data were collected at a resolving power of 17,500 (FWHM *m/z* 200) in negative polarity; see Figure 3. The Alkylphenol ethoxylates were determined using Single Ion Monitoring (SIM) where MS1 data were collected at a resolving power of 70,000 (FWHM *m/z* 200) in positive polarity.

Figure 2. Q Exactive Focus Mass Spectrometry showing Equan On-Line SPE System.

LCMS Analysis

Method Calibration

All calibration standards were prepared in LCMS grade water with 10% LCMS grade methanol; 2.5 mL volumes were used for analysis and the calibration was carried out using internal standardisation for the APs and Bisphenol A and external standardisation for the APEOs. Calibrants were prepared at between 10 and 1200 ng/L, as appropriate for each target.

Acquisition, Processing and Confirmation

The data were acquired, processed and confirmed using Thermo Scientific™ TraceFinder™ 4.1 software. Data confirmation using the accurate mass of the MS1 and MS2 Target and Confirming ions respectively; as well as the isotopic pattern of MS1 ions for APEOs determined by SIM.

UKAS Method Validation

The methods were validated in accordance with the United Kingdom Accreditation Service (UKAS) guidelines, using the following water types:

- Waste Water Treatment Works (WWTW) effluent: A works based in Glasgow with a population equivalent of > 200,000.
- Ground Water was supplied by AQUACHECK (UKAS).
- Marine (saline) water was from the Scottish Ayrshire coast.
- River water was sampled from the River Clyde, Lanarkshire, Scotland.

The method detection limits (MDL) were derived according to NS304 documentation and procedures.

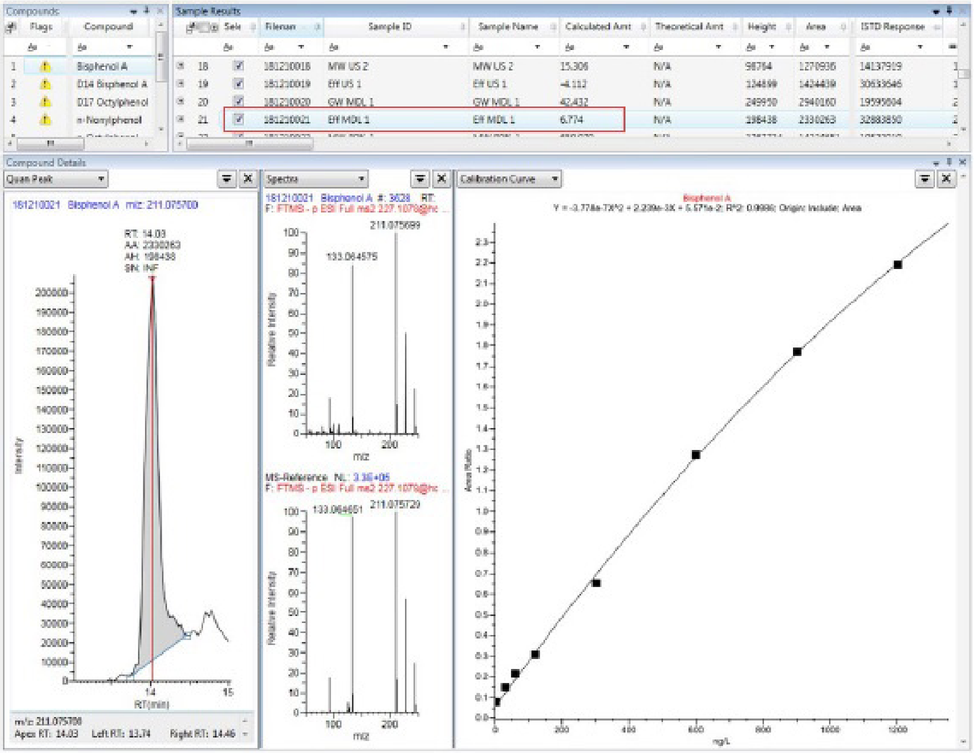
To derive the required statistics, 11 Validation batches comprising of duplicate samples were analysed. Matrix samples were spiked at a concentration which would give the minimum guaranteed response at the detector; appropriate blanks were also ran. To calculate the method detection limits, the within batch standard deviation was multiplied by a factor of 4.65. Method performance with respect to bias and precision was assed using representative clean and dirty matrix types; in this case ground water and WWTW effluent. To monitor routine operational performance, Quality Control matrix spikes at 10% and 90% of calibration range where employed along with unspiked matrix samples for blank correction purposes; lastly limits for precision and bias were set at 12.5% and 25% respectively.

RESULTS

Figures 3 to 6 show typical calibration curves for bisphenol A, nonylphenol, nonylphenol mon- ethoxylate and t-octylphenol.

Also shown are chromatograms of procedural target spikes at concentrations close to the method detection limits, in the various matrices described in the UKAS Method Validation section.

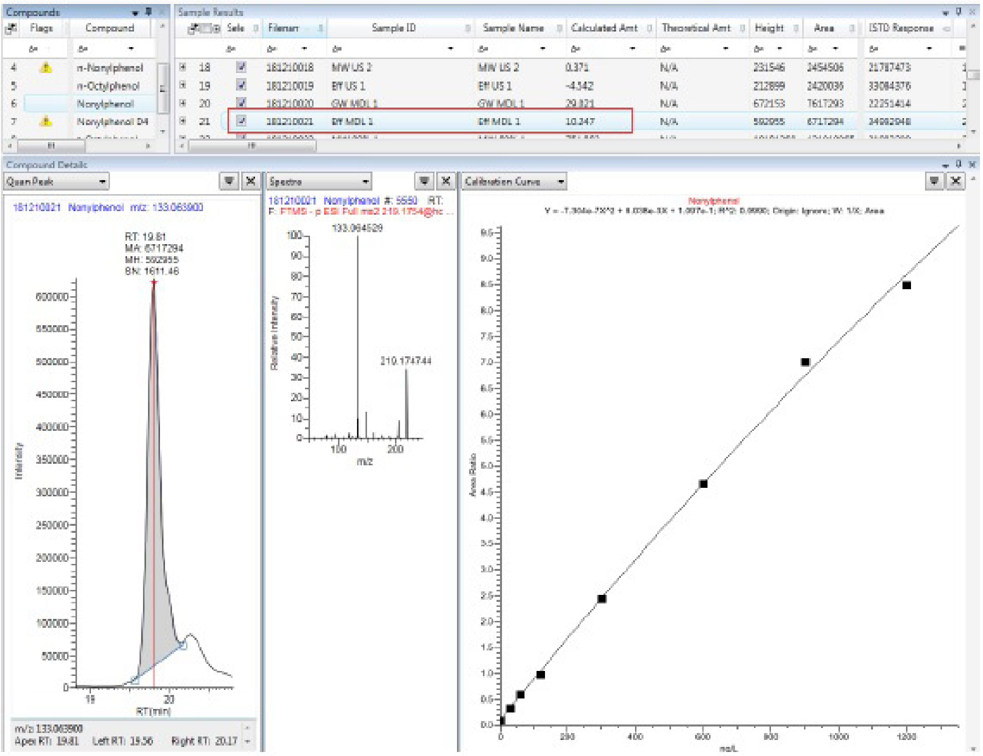
Due to space limitations, the other targets are not shown but relevant data are tabulated in the Abstract Section.



	Flags	Compound
1	⚠	Bisphenol A
2	⚠	D14 Bisphenol A
3	⚠	D17 Octylphenol
4	⚠	n-Nonylphenol

	Sele	Filenam	Sample ID	Sample Name	Calculated Amt	Theoretical Amt	Height	Area	ISTD Response
18	☑	181210018	MW US 2	MW US 2	15.306	N/A	98764	1270936	14137919
19	☑	181210019	Eff US 1	Eff US 1	-4.112	N/A	124899	1424439	30633646
20	☑	181210020	GW MDL 1	GW MDL 1	42.432	N/A	249950	2940160	19595604
21	☑	181210021	Eff MDL 1	Eff MDL 1	6.774	N/A	198438	2330263	32883890

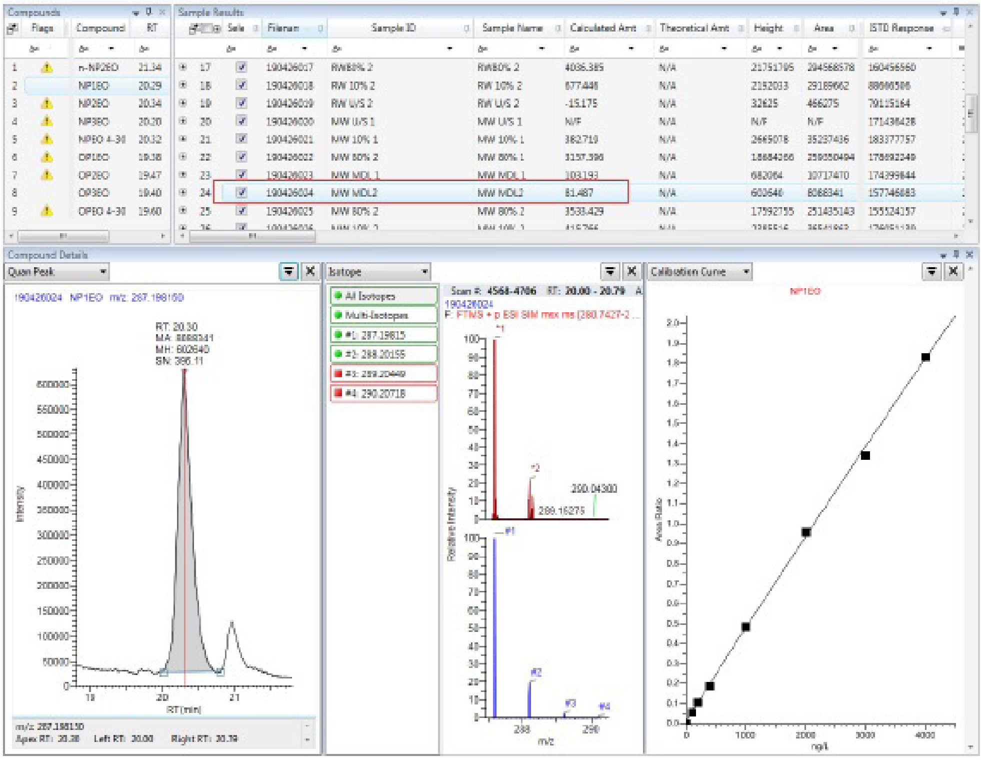
Figure 3. Bisphenol A Internal Calibration, showing chromatogram of spike near MDL in waste effluent. Negative mode PRM.



	Flags	Compound
4	⚠	n-Nonylphenol
5		n-Octylphenol
6		Nonylphenol
7	⚠	Nonylphenol D4

	Sele	Filenam	Sample ID	Sample Name	Calculated Amt	Theoretical Amt	Height	Area	ISTD Response
18	☑	181210018	MW US 2	MW US 2	0.371	N/A	231546	2454506	21787473
19	☑	181210019	Eff US 1	Eff US 1	-4.342	N/A	212899	2420036	33084376
20	☑	181210020	GW MDL 1	GW MDL 1	29.821	N/A	672153	7617293	22251414
21	☑	181210021	Eff MDL 1	Eff MDL 1	10.247	N/A	592955	6717294	34992948

Figure 4. Nonylphenol Internal Calibration, showing chromatogram of spike near MDL in waste effluent. Negative mode PRM.



Compounds

	Flags	Compound	RT
1	⚠	n-NP2EO	21.34
2		NP1EO	20.29
3	⚠	NP2EO	20.34
4	⚠	NP3EO	20.20
5	⚠	NPEO 4-30	20.32
6	⚠	OP1EO	19.38
7	⚠	OP2EO	19.47
8		OP3EO	19.40
9	⚠	OPEO 4-30	19.60

Sample Results

	Sele	Filenam	Sample ID	Sample Name	Calculated Amt	Theoretical Amt	Height	Area	ISTD Response
17	☑	190426017	RW80% 2	RW80% 2	4036.385	N/A	21751795	204568578	160456560
18	☑	190426018	RW 10% 2	RW 10% 2	677.448	N/A	2152033	29189662	88686598
19	☑	190426019	RW U/S 2	RW U/S 2	-15.175	N/A	32625	466275	79115164
20	☑	190426020	MW U/S 1	MW U/S 1	N/F	N/A	N/F	N/F	171436428
21	☑	190426021	MW 10% 1	MW 10% 1	382.719	N/A	2665078	35237436	183377757
22	☑	190426022	MW 80% 2	MW 80% 1	3157.396	N/A	18684266	259050494	178692249
23	☑	190426023	MW MDL 1	MW MDL 1	103.193	N/A	682064	10717470	174399844
24	☑	190426024	MW MDL2	MW MDL2	81.487	N/A	602640	8088341	157746683
25	☑	190426025	MW 80% 2	MW 80% 2	3538.429	N/A	17592755	251435143	155524157

Compound Details

Quan Peak

190426024 NP1EO m/z: 287.198150

RT: 20.30
MA: 8088341
MH: 602640
SN: 386.11

m/z: 287.198150
Apex RT: 20.30 Left RT: 20.00 Right RT: 20.79

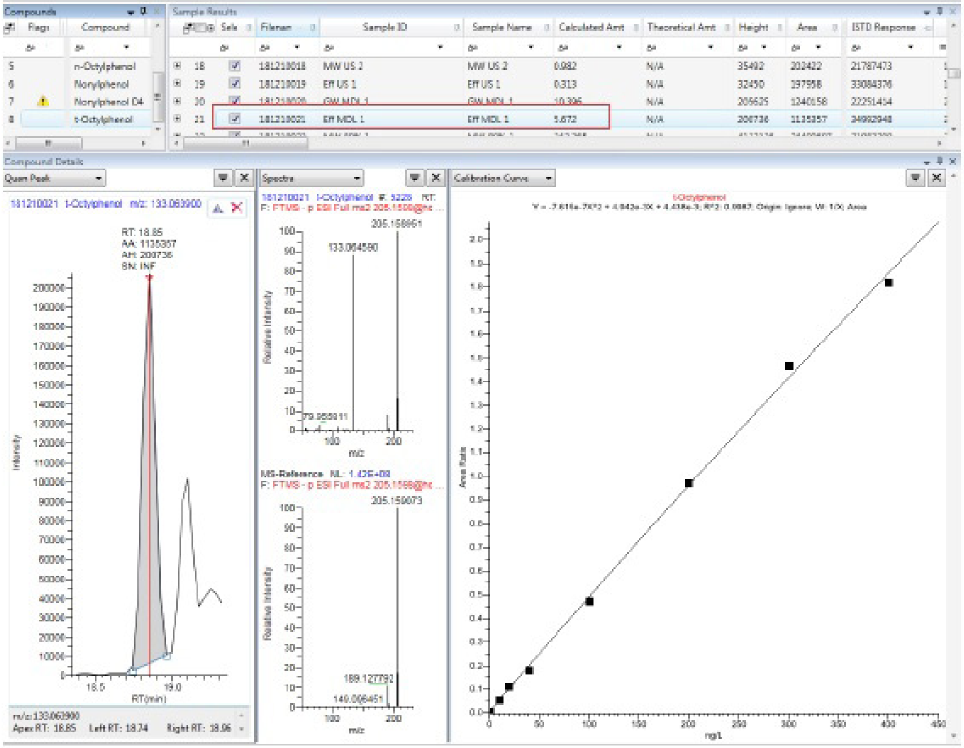
Isotope

- ● All Isotopes
- ● Multi-Isotopes
- ● #1: 287.19815
- ● #2: 288.20155
- ■ #3: 289.20449
- ■ #4: 290.20718

Scan #: 4568-4706 RT: 20.00 - 20.79
190426024
F: FTMS + p ESI SIM msx ms [280.7427-2 ...

Calibration Curve

Figure 5. Nonylphenol Monoethoxylate Calibration, showing chromatogram of spike near MDL in Marine Water. Positive mode SIM.



Compounds

	Flags	Compound
5		n-Octylphenol
6		Nonylphenol
7	⚠	Nonylphenol D4
8		t-Octylphenol

Sample Results

	Sele	Filenam	Sample ID	Sample Name	Calculated Amt	Theoretical Amt	Height	Area	ISTD Response
18	☑	181210018	MW US 2	MW US 2	0.982	N/A	35492	202422	21787473
19	☑	181210019	Eff US 1	Eff US 1	0.313	N/A	32450	197958	33084376
20	☑	181210020	GW MDL 1	GW MDL 1	10.396	N/A	205625	1240158	22251454
21	☑	181210021	Eff MDL 1	Eff MDL 1	5.672	N/A	200736	1135357	34992948

Compound Details

Quan Peak

181210021 t-Octylphenol m/z: 133.063900

RT: 18.85
AA: 1135357
AH: 200736
SN: INF

m/z: 133.063900
Apex RT: 18.85 Left RT: 18.74 Right RT: 18.96

Spectra

181210021 t-Octylphenol #: 5228 RT:
F: FTMS - p ESI Full ms2 205.1500@hc ...

MS-Reference NL: 1.42E+08
F: FTMS - p ESI Full ms2 205.1500@hc ...

Calibration Curve

t-Octylphenol
Y = -7.616e-7X^2 + 4.842e-3X + 4.438e-3; R^2: 0.9982; Origin: Ignore; W: 1/X; Area

Figure 6. t-Octylphenol Internal Calibration, showing chromatogram of spike near MDL in waste effluent. Negative mode PRM.

DISCUSSION/CONCLUSIONS

- Using on-line SPE, with PRM (negative) and SIM (positive) acquisition for bisphenol A, the alkylphenols and related ethoxylates, the results have shown excellent method performance both in terms of quantitation and confirmation, and in speed of analysis compared to traditional approaches.
- Through rigorous validation using a wide range of representative sample types (waste water, ground water, river water and marine water), the methods have been shown to meet the stringent method detection limits required to comply with the EU Water Framework Directive and related Environmental Quality Standards.
- If the duration of current methods (see Figure1) is assumed to be in the order of 10 hours, then the method described is approximately 30 times faster, which has implications in terms of sampling logistics, capital expense and maintenance, as well as expense of consumables.

REFERENCES

1. Crisp, T. M. et al. Environmental endocrine disruption: an effects assessment and analysis. *Environ. Health Perspect*. 106, 11–56 (1998).
2. Ahel, M., Giger, W. & Koch, M. Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment—I. Occurrence and transformation in sewage treatment. *Water Res*. 28, 1131–1142 (1994).
3. EU Water Frame Work Directive. http://ec.europa.eu/environment/water/water-framework/index_en.html
4. NS30 - A manual on analytical quality control for the water industry. Cheeseman R.V., Wilson A.L.1989.

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